Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 26, 28, 33, 117-125, and 127-151 are pending in the application, with claim 26 being the independent claim. Claims 26, 28, and 117-148 have been amended and claims 149-151 have been added. Support for these amendments can be found at least in the cancelled claims and throughout the specification, for example, on page 23, lines 7-20; page 10, table 7; page 99, lines 9-18; and page 101, lines 13-21. It is believed these changes introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Interview

Applicants thank Examiner Nashed for the courteous and helpful interview held with Applicants' undersigned representative on May 23, 2003.

The Sequence Listing

The Office Action, at page 2, maintained that the present application is not in compliance with the sequence rules. The concern expressed in the Office Action is that the specification describes mutations at several specific positions in a protein without describing

the sequence of the protein, and that the sequence in a database may change. (Paper 20, p. 2.) Applicants respectfully disagree with the objection.

Applicants need not disclose art-known or standard amino acid sequences or the positions of conserved residues. As the PTO and the courts have stated, what is known in the art need not be disclosed.

At page 57, the specification describes mutations at positions Asp450 and Asp505 in the RNAse H domain of RSV reverse transcriptase. These positions correspond to conserved residues that were described, for example, in Johnson *et al.*, *PNAS USA 83*:7648-7652 (1986) (IDS Document AT18). Johnson *et al.* published an alignment of RSV, M-MLV, HIV and other reverse transcriptase sequences in 1986, which showed that reverse transcriptases have significant conservation between a 150-residue segment in their carboxyl termini (the RNase H domain) and a 250 residue segment in their amino termini (the polymerase domain). *Id.* p. 7649-50, figures 2-4. Johnson *et al.* also pointed out residues that are conserved between the sequences and identified consensus sequences and motifs. *Id.* One of the sequences aligned in Johnson *et al.* was that of RSV reverse transcriptase. The alignment of the RNAse H domain in Figure 2 of Johnson *et al.* shows both Asp450 and Asp505 of RSV reverse transcriptase, which are marked with asterisks because they are conserved across the aligned sequences. Therefore, the sequence of the RSV reverse transcriptase that was mutanted as described at page 57 need not be disclosed in the sequence listing.

At page 73, the specification makes reference to RSV and AMV reverse transcriptases by their GenBank accession numbers. Assuming, arguendo, that changes in a database sequence are even an issue, the artisan can consult the publications cited in the

databases, see entries for the GenBank accession numbers referred to in the specification[1], or can consult the revision history[2] and the "dead" entries listed therein. Therefore, the sequences of the RSV and AMV reverse transcriptases referred to on page 73 by GenBank accession number need not be disclosed in the sequence listing.

Accordingly, withdrawal of the objection is respectfully requested.

The Rejection Under 35 U.S.C. § 112, First Paragraph Is Traversed

The Office Action, at page 3, maintained the rejection of claims 26, 28, 33, 117-125, and 127-148 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention. Applicants respectfully traverse this rejection.

1. The Deposited Biological Material

The Office Action states that the deposited material, if perfected, would overcome the rejection as to claims directed to the wild-type homodimer and heterodimer, and the specific deposited mutants.

Applicants submit herewith a copy of a Declaration Concerning Deposited Biological Material, filed in related Application No. 09/245,026 on October 22, 2002. Applicants therefore respectfully believe all requirements concerning the deposited material have been met. 37 C.F.R. 1.801-1.809. Accordingly, withdrawal of this portion of the rejection is respectfully requested.

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[1] Copies submitted herewith for the convenience of the Examiner.

[2] Copies submitted herewith for the convenience of the Examiner.

2. The RNase H Domain

The Office Action also states that the specification fails to teach the RNase H domain and residues that affect its activity. Applicants respectfully traverse.

As mentioned above, Johnson *et al.* published an alignment of RSV, M-MLV, HIV and other reverse transcriptase sequences in 1986 and determined that reverse transcriptases have significant conservation between a 150-residue segment in their carboxyl termini, which is the RNase H domain. Johnson, M.S. *et al. PNAS USA 83*:7648-7652 (1986), p. 7649-50, figures 2-4 (IDS Document AT18). Johnson *et al.* also noted the significant conservation between *E. coli* RNAse H and the RNAse H domain of reverse transcriptases. *Id.*, p. 7649, fig. 1. The crystal structures for *E. coli* RNAse H and HIV-1 RNAse H have been determined, confirming that they contain highly related structures. Volkmann; S. *et al.*, *J. Biol. Chem. 268*:2674-2683, p. 2675, col. 1 (1993).[3]

Additionally, Johnson *et al.* pointed out residues within the RNAse H domain that are conserved among the reverse transcriptase sequences they aligned. *Id.* For example, the authors identified residues D450, Q481, E483, L492, N501, D505, S506, H549, N560, and D564 of the RNAse H domain, corresponding to RSV reverse transcriptase, as being identical across the aligned sequences. *Id.*, p. 7649, fig. 2. Others subsequently determined that seven of the amino acids identified by Johnson *et al.* are conserved among retroviral and bacterial sequences and two are invariant among the then known reverse transcriptase sequences. Volkmann *et al.*, p. 2681, col. 1

Moreover, mutations have been made in certain conserved residues that establish which residues are essential for RNAse H activity. The two invariant residues have been

[3] Copy enclosed herewith for the convenience of the Examiner.

mutated, creating N494D and Q475E of HIV-1 reverse transcriptase. Volkmann *et al.*, p. 2674, abstract. N494D is similar to the wild-type form in activity. *Id.* This residue is not located in the RNAse H active center, and probably is not involved in substrate binding, but may contribute to tertiary stability. *Id.*, p. 2682, col. 2. On the other hand, Q475E was defective in activity, and probably directly contacts the RNA-DNA substrate. *Id.*, p. 2674, abstract, and p. 2682, col. 2. It has also been determined that D443 and D498 of HIV-1 reverse transcriptase are part of the active center of RNAse H, and that H539 appears to be required for RNA-DNA substrate binding. *Id.*, p. 2675, col. 1.

Therefore, the conserved residues of the RNAse H domain were known, and need not have been disclosed in the present specification. *See Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d at 1384, 231 USPQ at 94; MPEP 2163, p. 2100-165, col. 2 (Rev. 1, Feb. 2003). Accordingly, withdrawal of this portion of the rejection is respectfully requested.

3. Monomeric Form and Multimeric Forms

The Office Action also states that the specification fails to teach a polymerase activity for any form of AMV reverse transcriptase other that a dimer, and that no one has taught a monomeric form of AMV reverse transcriptase. Applicants respectfully traverse.

About 30 years ago, Grandgenett *et al.*, showed that AMV reverse transcriptase is active in the monomeric α form. Grandgenett, D.P., *et al.*, *Proc. Natl. Acad. Sci. USA* *70*:230-234 (1973) (IDS Document AT5). The authors stated that the monomeric α form contained both RNA-dependent DNA polymerase activity and RNase H activity. *Id.*, abstract, p. 230. Thus, the Office Action is in error.

With regards to multimeric forms, Applicants have amended the claims to cancel the language "at least one subunit" and "one or more" subunits. Accordingly, withdrawal of this portion of the rejection is respectfully requested.

4. βp4 Subunit

Regarding the term "βp4 subunit," Applicants described the βp4 subunit throughout the specification. As the specification says,

> Various forms of the individual subunits of ASLV RT have been cloned and expressed. These include a 98-kDa precursor polypeptide that is normally processed proteolytically to β and a 4-kDa polypeptide removed from the β carboxy end (Alexander, F., *et al., J. Virol. 61*: 534 (1987) and Anderson, D. *et al., Focus 17*:53 (1995)), and the mature β subunit (Weis, J.H. and Salstrom, J.S., U.S. Patent No. 4, 663, 290 (1987); and Soltis, D.A. and Skalka, A.M., *Proc. Nat. Acad. Sci. USA 85*:3372 (1988)).

Specification, p. 4, lines 22-28. The specification also describes the construction of a gene encoding the mature β subunit by inserting a translational stop codon at the "p4" subunit cleavage site. *See,* p. 56, lines 12-14. As Alexander *et al.* proposed, the β subunit is initially synthesized as a larger precursor that is cleaved to produce the smaller mature form and a 4 kD fragment. Alexander, F., *et al., J. Virol. 61*: 534 (1987), p. 540, figure 6 (IDS Document AT1). Thus, based on the specification and the well known structure of the β subunit precursor, one of ordinary skill in the art would understand that the "βp4 subunit" is the precursor that contains the mature β subunit and the 4 kD fragment. Additionally, mutations in the βp4 subunit are disclosed, for example, at page 20, line 15 to page 22, line 16; and Examples 1 and 7 (pp. 57 and 91-2, and 95). Thus, the specification conveys the structure of the βp4 subunit to one of ordinary skill in the art.

Regarding the activity of the βp4 subunit, the specification shows that dimeric βp4 (βp4βp4) RSV reverse transcriptase does have activity. Specification, p. 102, Table 7. Table 7 shows that the βp4βp4 form has approximately the same level of activity as the ββ form. RSV and AMV are both part of the ASLV, or alphavirus, genus of retroviruses, and their reverse transcriptases have the same subunit and domain structure. *See, e.g.*, Dimcheff, *et al.*, *J. Virology 75*:2002-2009 (2001), p. 2002, col. 1, lines 4-7[4]; The Universal Virus Database of the International Committee on Taxonomy of Viruses, <http://www.ictvdb.iacr.ac.uk/Ictv/fs_retro.htm>[5]; *and* Prasad V.R., "8. Genetic Analysis of Retroviral Reverse Transcriptase Structure and Function," in: *Reverse Transcriptase*, Skalka, A.M. and Goff, S.P., eds., Cold Spring Harbor Laboratory Press, Plainview, NY, pp. 135-162, at p. 135 (1993) (IDS document AR13). One of ordinary skill would recognize evidence of the activity of the βp4βp4 form of RSV reverse transcriptase as being evidence of the activity of the βp4βp4 form of AMV reverse transcriptase. Accordingly, withdrawal of this portion of the rejection is respectfully requested.

The Rejections Under 35 U.S.C. § 112, Second Paragraph Are Traversed

The Office Action, at page 4, maintained the rejection of claims 26, 28, 33, 40, 117-125, and 127-148 under 35 U.S.C. § 112, second paragraph as allegedly being indefinite. Applicants respectfully traverse this rejection.

With regards to the recited "polymerase activity," Applicants have amended the claims as suggested by the Examiner. Accordingly, this portion of the rejection is moot.

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[4] Copy enclosed herewith for the convenience of th Examiner.

[5] Copy enclosed herewith for the convenience of the Examiner.

With regards to the recitation of "one or more subunits," the Office Action is mistaken in the statement that no monomers of AMV reverse transcriptase have activity. As discussed above, Grandgenett *et al.* found that the α monomeric form of AMV reverse transcriptase has RNA-dependent DNA polymerase activity. Nevertheless, Applicants have amended the claims to cancel the language the Examiner found objectionable, merely to expedite prosecution. Accordingly, this portion of the rejection is moot.

With regards to the term "βp4 subunit," the Office Action is correct in the statement that this term refers to the precursor that produces the β subunit. However, the Office Action is incorrect in the statement that the β subunit is formed by cleavage from the α subunit. In fact, the opposite is true: the α subunit is formed by cleavage from the β precursor subunit. As the specification states, ALSV reverse transcriptase is "a heterodimer of two subunits, α (approximately 62 kDa) and β (approximately 94 kDa), in which α is derived from β by proteolytic cleavage." Specification, p. 3, line 27 to p. 4, line 10.

The specification also states:

> Various forms of the individual subunits of ASLV RT have been cloned and expressed. These include a 98-kDa precursor polypeptide that is normally processed proteolytically to β and a 4-kDa polypeptide removed from the β carboxy end (Alexander, F., *et al., J. Virol. 61*: 534 (1987) and Anderson, D. *et al., Focus 17*:53 (1995)), and the mature β subunit (Weis, J.H. and Salstrom, J.S., U.S. Patent No. 4,663, 290 (1987); and Soltis, D.A. and Skalka, A.M., *Proc. Nat. Acad. Sci. USA 85*:3372 (1988)).

Specification, p. 4, lines 22-28. The specification also describes the construction of a gene encoding the mature β subunit by inserting a translational stop codon at the "p4" subunit cleavage site. *See* specification, p. 56, lines 12-14. As Alexander *et al.* proposed, the β subunit is initially synthesized as a larger precursor that is cleaved to produce the mature

form and a 4 kD fragment. Alexander, F., *et al., J. Virol. 61*: 534 (1987), p. 540, figure 6 (IDS Document AT1). Thus, based on the specification and the well known structure of the β subunit precursor, one of ordinary skill in the art would understand that the "βp4 subunit" is the precursor that contains the mature β subunit and the 4 kD fragment. Thus, the term is clear and definite to one of ordinary skill in the art. Accordingly, withdrawal of this portion of the rejection is respectfully requested.

The Rejection Under 35 U.S.C. § 102(b) Is Traversed

The Office Action, at page 6, maintained the rejection of claims 26, 28, 33, 40, 117-119, 121-125, and 127-148 under 35 U.S.C. § 102(b) as allegedly being anticipated by Soltis *et al.* (*Proc. Natl. Acad. Sci. USA 85*:3372-76 (1988)). Applicants respectfully traverse this rejection.

The Office Action stated that the rejection would be withdrawn if the claims were amended to recite "RNA-dependent DNA" polymerase specific activity. Applicants have amended the claims as suggested by the Examiner. Accordingly, withdrawal of the rejection is respectfully requested.

The Rejection Under 35 U.S.C. § 103(a) Is Traversed

The Office Action, at pages 7-8, maintained the rejection of claims 26, 28, 33, 40, 117-119, 121-125, and 127-148 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Soltis *et al.* in view of the state of the art at the time the application was filed. Applicants respectfully traverse this rejection.

To establish a prima facie case of obviousness, the prior art must teach or suggest all the claim limitations and there must be a reasonable expectation of success. *Smiths Indus. Med. Sys. v. Vital Signs, Inc.*, 183 F.3d 1347, 1356, 51 USPQ2d 1415, 1420 (Fed. Cir. 1999). The prior art *as a whole* must be considered in an obviousness analysis. *In re Young*, 927 F.2d 588, 10 USPQ2d 1089 (Fed. Cir. 1991); MPEP 2143.01, p. 2100-126, col. 1. Moreover, the mere fact that references could be combined, or that the modifications were within the level of ordinary skill, is irrelevant absent an objective motivation to combine or modify the teachings. *In re Mills*, 916 F.3d 680 (Fed. Cir. 1990); *In re* Kotszab, 217 F.3d 1365, 1371 (Fed. Cir. 2000); Ex *parte Levengood*, 28 USPQ2d 1300 (Bd. Pat. App. & Inter. 1993).

Soltis *et al.* disclose the independent expression in *E. coli* of the AMV reverse transcriptase α and β subunits, and the purification of each to a specific activity 10,000-100,000 fold *lower* than the specific activity of AMV reverse transcriptase purified directly from AMV. Soltis et al. also disclose the expression and purification of the full length *pol* product in *E. coli*.

The pending claims are directed to a method of producing AMV reverse transcriptase by expressing it in a eukaryotic cell, and isolating or purifying the expressed reverse transcriptase, with the resulting AMV reverse transcriptase having a specific activity of at least about 30,000 units per milligram. Thus, the present claims recite (1) expression in eukaryotic cells, (2) of AMV enzyme, (3) having a specific activity of ≥ 30,000 units per milligram. Soltis *et al.* do not teach or suggest all three limitations. Further, there was no motivation to modify Soltis *et al.* based on knowledge in the art as exemplified by Ford *et al.*, *Prot. Express. Purif.* 2:95-107 (1991), as cited by the Examiner.

The priority application for the present application was filed April 22, 1997. In 1997, the artisan had no motivation to produce recombinant AMV reverse transcriptase because *non-recombinant* AMV reverse transcriptase with a very high specific activity was already available by 1995. The Boehringer Mannheim catalog, pages 92-93 (1995) (IDS Document AR17), lists an AMV reverse transcriptase *purified from AMV* that has a specific activity of >50,000 units per milligram.

Moreover, Applicants assert that when the art *as a whole* is considered, not only would there have been no motivation to lead the artisan of ordinary skill to modify Soltis *et al.* to obtain the presently claimed invention, but there would not have been a reasonable expectation of successfully obtaining the presently claimed invention. Unlike the situation for other reverse transcriptases, there were difficulties associated with attempts to produce AMV reverse transcriptase (and other ASLV reverse transcriptases) via recombinant methods. For example, Prasad, V.R., in a review article, stated,

> The avian retroviral RT was the first enzyme to be purified to homogeneity and characterized biochemically; it has become the staple of many recombinant DNA procedures because it is readily available in very high quantities from avian myeloblastosis virus (AMV) particles. However, the genetics of the avian RT has lagged behind that of the mammalian enzymes. It is, in general, more difficult to transfect and clone avian cells than mammalian cells, and thus it has been harder to generate mutant producer cell lines. *The more complex subunit structure of the avian RT has also inhibited its preparation through recombinant DNA methods.* Despite these difficulties, considerable information has been obtained concerning this RT.

Prasad, paragraph spanning pp. 138-139 (emphasis added) (IDS document AR13). Thus, even though reverse transcriptase from AMV was isolated and characterized before any mammalian reverse transcriptases, it was not produced at high specific activity levels by

recombinant methods until the present invention was made – well after mammalian enzymes such as MMLV reverse transcriptase and HIV-1 reverse transcriptase, which were first produced recombinantly in 1985 and 1987, respectively. Prasad, page 140, first paragraph, and page 144, second paragraph.

Further, the art actually taught away from the claimed invention. For example, Kawa, S. *et al., Prot. Expression and Purification 4*:298 (1993) (IDS document AR8) disclose the expression of HIV reverse transcriptase in insect cells to obtain an enzyme with a specific activity of 103 units per milligram.[6] The authors also report that HIV reverse transcriptase produced in *E. coli* has a specific activity of 160 units per milligram. Thus, the method taught in Kawa *et al.*, using expression in insect cells, produced an enzyme with lower specific activity than a method using expression in *E. coli*.

Likewise, the art discloses that HIV-1 reverse transcriptase produced in yeast has a lower specific activity than that produced in *E. coli*. For example, Barr *et al.* produced HIV-1 reverse transcriptase in yeast, and obtained enzyme with a specific activity of 34,666 units per milligram. Barr, P.J. *et al., BioTechnology 5*:486 (1987) (IDS document AT2). Lowe *et al.* produced HIV-1 reverse transcriptase in *E. coli*, and obtained enzyme with a specific activity of 1,610 units per milligram. Lowe, D.M. *et al., Biochemistry 27*:8884 (1988) (IDS document AR11). However, Barr *et al.* and Lowe *et al.* used different definitions for

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[6] A unit is defined therein as the "amount of enzyme required to catalyze the incorporation of 1 nmol of dTMP in *1 minute* at 25°C using poly(A)-oligo$(dT)_{16}$ as template-primer." p. 302, first paragraph (emphasis added). This is the equivalent of 1030 units per milligram, when a unit is defined as the amount enzyme required to catalyze the incorporation of 1 nmol of dTMP in *10 minutes* at 25°C. At 37°C, the specific activity would be higher.

specific activity.[7] When the specific activity of each is adjusted to define a unit as the amount of enzyme required to incorporate 1nmol dTMP at 37°C in *10 minutes*, as defined in the present specification, then the following results were obtained by Barr *et al.* and Lowe *et al.*:

Host cell	Specific Activity (units per milligram)
Yeast	5,778
E. coli	16,1000

Therefore, the art taught away from expressing reverse transcriptases in eukaryotic cells such as insect cells or yeast cells to obtain a reverse transcriptase with high specific activity.

In summary, Applicants respectfully submit that there was no motivation to produce recombinant AMV reverse transcriptase because non-recombinant enzyme having very high specific activity was already available. Further, even if there had been a motivation to produce recombinant AMV reverse transcriptase, there was no reasonable expectation of successfully obtaining recombinant enzyme with high specific activity because of the complex structure of the AMV enzyme. Moreover, the art taught away from the present claims, which recite a method of production in eukaryotic cells to obtain an AMV enzyme having a specific activity of at least about 30,000 units per milligram. Therefore, the claimed invention is non-obvious over the prior art.

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[7] Barr *et al.* defined a unit as the amount of enzyme required to incorporate 1 nmol of dTMP in *60 minutes* at 37°C. Barr *et al.*, page 488, table 1. Lowe *et al.* defined a unit as the amount of enzyme required to incorporate 1 nmol of dTMP in *1 minute* at 37°C. Lowe *et al.*, page 8886, table 1.

During the interview, the Examiner questioned the applicability of the specific activity of RSV reverse transcriptase described in the specification to the claims, which recite AMV reverse transcriptase. As discussed above on page 16, one of ordinary skill would recognize evidence concerning RSV reverse transcriptase as being evidence concerning AMV reverse transcriptase. Nonetheless, Applicants also provide Gerard, G.F *et al.*, *Nucleic Acids Research 30*:3118-3129 (2002)[8] as additional evidence regarding the specific activity of AMV reverse transcriptase produced by the method of the invention. On page 3119, second column, the authors describe the purification and characterization of AMV reverse transcriptase produced in insect cells. As noted, the recombinant enzyme had a specific activity of 57,500 units per milligram. Thus, the claimed method produces both RSV reverse transcriptase and AMV reverse transcriptase having a specific activity of at least about 30,000 units per milligram.

Applicants therefore respectfully assert that the present method is non-obvious over the prior art. Accordingly, withdrawal of this rejection is respectfully requested.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance.

[8] Copy submitted herewith for the convenience of the Examiner.

If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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